The recent advances of CAR T cell immunotherapy

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Abstract: Harnessing patient's own immune system to fight against cancers is a permanent goal of tumor immunotherapy. Chimeric antigen receptor (CAR) T cell therapy is emerging as a promising way in treating leukemia and solid tumors. Now, two commercial CAR T therapies have been approved by the US Food and Drug Administration for treating patients with B cell lymphomas. However, continuous efforts are still need to increase the efficacy of CAR T cells in curing cancers, and especially to improve their activity in solid tumors. This review summarizes the novel design of CAR structure and many other measures in improving the anti-tumor activity and overcoming immunosuppressive tumor microenvironment, and reducing toxicities.

1. Introduction

The chimeric antigen receptor (CAR)-T cell immunotherapy, is using and genetically modifying autologous T cells to treat patients' own leukemia and solid tumors [1]. The CAR is composed of single-chain fragment variable (scFv), which functions to recognize tumor specific antigens, a transmembrane domain (TM), and an intracellular domain (ICD) for signaling transduction [2]. scFv is mainly derived from variable region of light and heavy chain of antibody backbone, which is connected by a glycine/serine-rich flexible linker. The scFv, similar to antibody, can recognize and bind to a specific antigen. The hinge and transmembrane domain, mainly derived from CD28, is a hydrophobic region that connecting the scFv and the intracellualr stimulatory domains [3]. The intracellular domain is composed of multiple T-cell activation domain and co-stimulatory domain, such as CD3ζ, ICOS, 4-1BB and so on [4].

The CAR encoding transgenes are introduced to isolated autologous T lymphocytes mainly through gamma-retrovirus or lentivirus based transduction. The CAR T cells after transduction will be expanded for weeks to sufficient numbers before re-infusing into the patient's body [5]. The infused CAR T cells will recognize tumor-specific antigen and act as "living drugs" that may exert both immediate and long-term effects. Immediate effects include secretion of large quantities of cytokine, proliferation and activation of CAR T cells and direct lysis of the tumor cells. Clinical trials have demonstrated the potent activity of anti-CD19 CAR T cells against multiple subtypes of B-cell lymphoma [6, 7]. In 2017, the Food and Drug Administration (FDA) approved Novartis' Kymriah and Kite's Yescarta, the first two anti-CD19 CAR T therapy for acute lymphoblastic leukemia. In this review, we mainly focus on the recent advances of CAR T development in improving the anti-tumor activity and safety, and also the studies of CAR T in solid tumors.

2. Different modifications in chimeric antigen receptor (CAR) design

Chimeric antigen receptors (CAR) are artificial designed transmembrane receptors which include scFv, TM and ICD. The ICD combined scFv with various signaling domain from costimulatory protein, such as CD28, ICOS or 4-1BB. It was demonstrated that this signaling domain is of vital important to the activity of CAR T cells [8].

Most of the studies and clinical trials used unselected "bulk" T cells, which contains both CD4+ and CD8+ T cells, to generate CAR T cells. Though CD4+ T cells do not directly kill tumor cells, they provide various cytokines and important costimulatory signals to the CD8+ populations, augmenting the killing effect of cytotoxic effector CD8+ CAR T cells. Recently, Guedan and colleagues showed that CARs with ICOS and 4-1BB ICD dramatically enhanced the in *vivo*

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persistence of CD4+ CAR T cells that, in turn, increased the persistence of CD8+ CAR T cells [9]. CAR T cells combining ICOS and 4-1BB signaling domain (ICOSBBz) exhibited superior anti-tumor capacity during cocultured with mesothelin-expressing tumor cell lines. Although there is lower surface expression of ICOSBBz CAR in T cells, they exhibited more potency in inhibiting tumor growth. This research also demonstrated that ICOSBBz-CAR T cells not only maintain 100% tumor regression within 35 days, but also shows an enhanced expansion rate compared with BBICOSz-CAR T cells. Moreover, CD4+ CAR T cells with ICOS signal played a beneficial role to enhance the persistence and killing effect of CD8+ CAR T cells in various solid tumor models.

Some cytokines can also significantly bolster the persistence and activity of CAR T cells. For example, IL-7 that secreted by T cells exhibits enhanced anti-tumor capacity. Active IL-7 receptors transmit signals by activating STAT5 downstream pathway. Shum and colleagues constructed a constitutively activating cytokine receptor C7R, and found C7R co-expressing CAR T cells showed increased proliferation, survival, and anti-tumor activity during repeated exposure to tumor cells, without T cell dysfunction or autonomous T cell growth [10]. In the GD2+ neuroblastoma tumor model, GD2-C7R CAR T cells showed enhanced secretion of IFN-γ and TNF-α when stimulated with tumors, the cytotoxicity and expansion rate of CAR T cells were also significantly improved. Moreover, the apoptosis rate of GD2-C7R CAR T cells also greatly reduced. Further study demonstrated that anti-apoptosis gene BCL2 was unregulated by C7R.

In another study, researchers constructed novel CAR molecules which containing truncated cytoplasmic domain from the interleukin (IL)-2 receptor β-chain (IL-2Rβ) and a STAT3-binding tyrosine-X-X-glutamine (YXXQ) motif, together with CD3ζ and CD28 co-stimulatory domains (28-ΔIL2RB-z(YXXQ)). The 28-ΔIL2RB-z(YXXQ) CAR T cells demonstrated activating JAK-STAT signals and high expression level of STAT3 downstream genes after antigen stimulation *in vitro*, and also showed significantly improved proliferation rate and less cell death upon antigen stimulation. The 28-ΔIL2RB-z(YXXQ) CAR T cells resulted in a longer survival time compared with 28-z and BB-z CAR T cells in NSG mice tumor model and NALM6-GL leukemic mice model [11].

3. Targeting antigens other than CD19

Anti-CD19 CAR T cells effectively mitigate various B cell leukemia, which is proved in clinical around the world. But, antigen loss is a critical reason causing the absent of long-term effectiveness and resistance to anti-CD19 CAR T therapy [12].

CD22 is also highly expressed in most leukemia, Fry and colleagues developed CAR T cell therapy that targeting to CD22. In a phase 1 clinical trial, anti-CD22 CAR T cells were used to treat B cell lymphoblast leukemia patients with relapsed or who is chemo-resistant. Of the 21 patients selected, 57% of patients achieved complete remission and some of them were minimal residual disease (MRD) negative. Some patients relapsed at 1.5-12 month after anti-CD22 CAR T infusion were associated with diminished CD22 surface expression. This is not unique to CD22-CAR T cell therapy, and also reflects that CAR T therapy required high level of antigen expression. Some patients who is resistant to anti-CD19 CAR T therapy retain CD22 expression, so, anti-CD19/CD22 bispecific CAR T cell could be more useful in this circumstance [13].

CD20 antigen is another well-established surface target for B-Non Hodgkin lymphoma (B-NHL), which is universally expressed with high density on surface of B cell lymphomas [14]. In an early phase IIa anti-CD20 CAR T clinical trial (NCT01735604), the researchers reported that, 4-6 weeks post-infusion, the overall response rate was 81.8%, with 54.5% of the patients (6/11) achieving a CR and 27.3% (3/11) achieving partial remissions (PRs). Moreover, this treatment regimen was well-tolerated, there was no grade four toxicities and no severe cytokine release syndrome been observed [15].

In view of the deficiency of single target of traditional CAR T, researchers designed a tandem bispecific CD19/CD20 CAR, which can induce activation of T cells by encountering tumor cells expression either CD19 or CD20 [16]. Co-culture experiment showed that the levels of IFN-γ, TNF-α, IL-2 and GM-CSF significantly increase after incubation of CAR T cells and tumor cell line.

In the Raji-NSG mouse tumor model, the CD19/CD20 CAR T cells showed more effective and less toxic effects [17].

CD123 is highly expressed on CD19-negative blasts within both bulk and leukemia initiating cell population. Moreover, CD123 is expressed on hematopoietic progenitor cells which may play a key role in resistance to chemotherapy and relapse after CAR T therapies. In an antigen loss, CD19-negative relapse xenograft model, the dual CD19/CD123 targeting CAR T cells can efficiently prevent CD19-negative tumor relapses, compared with CART19 monotherapy [18].

4. Improving CAR T-cell therapy effect

PD-1 blockade can also be used to boost the anti-tumor effect of CAR T therapy [19]. In one clinical trial, Chong and colleagues reported a case of refractory diffuse large B-cell lymphoma (DLBCL) patient that showed progressive lymphoma after receiving CART-19 therapy [20]. IHC staining indicated PD-L1 is highly expressed in patient's tumor biopsy. After treatment with Pembrolizumab, the size of patient's tumor significantly reduced. Those observations suggest that PD-1 blockade may enhance the efficacy of CAR T cells, especially in patients that failed to response to CAR T therapy before.

In 2016, Liu and colleagues applied CRISPR-Cas9 technology to simultaneously disrupt PD-1, T-cell receptor α constant (TRAC) and B2M genes in CAR T cells [21]. Compared with conventional CAR T cells, triple-negative CAR T cells lost PD-1, αβTCR and HLA-I expression on cell surface, which help to avoid the immunosuppressive effect of tumor microenvironment to CAR T cells, reduce the graft-versus-host-disease (GVHD) effect and immunogenicity of CAR T cells . In another study, researchers directly integrated CD19-specific CAR to the TRAC locus by using CRISPR-Cas9 gene editing method, which not only results in uniform CAR expression, but also enhances CAR T cell potency and delays T cell differentiation and exhaustion [22].

5. Development of CAR T immunotherapy in solid tumors

Although CAR T cell therapy has achieved great success in all kinds of leukemia, it has limited efficacy for solid tumors in clinical study. Several factors may contribute to the diminished function of CAR T cell for solid tumor, including insufficient traffic of CAR T cell to tumor sites, poor survival and persistence in solid tumor and immunosuppressive effects of the complex tumor microenvironment [23]. The tumor microenvironment is composed of immunosuppressive regulatory T cells, tumor associated macrophages cells and inhibitory metabolite, which will severely inhibit the infiltration and reduce the efficiency of anti-tumor function of CAR T cells [24].

Recently, Mata and colleagues developed an inducible activation of MyD88 and CD40 signaling system by fusing with the FKBP12 element in CAR T cells (iMyD88.CD40), which can be activated by a small-molecule drug [25]. This iMyD88.CD40 design not only greatly enhances effector function of CAR T cells, resulting in potent antitumor activity in preclinical solid tumors, but also provide a safety switch in controlling the activity of CAR T cells in solid tumors.

Previous studies demonstrated that CCL19 and IL7 produced by fibroblastic reticular cells are essential for the maintenance of T cells recruited in lymphoid organs. IL-7 could enhance the proliferation and survival of T cells, CCL17 is a chemokine to attract T cell and DCs. Recently, Adachi and colleagues engineered CAR T cells to express interleukin IL-7 and CCL19 (7×19 CAR T cells) [26]. Results demonstrated that CAR T cell expression IL7 and CCL19 induced massive T cells and DCs infiltration in tumor tissue, compared with conventional CAR T cells. In hCD20 mouse tumor model, treatment with anti-CD20 7×19 CAR T cells resulted in complete regression of tumor and long-term survival without tumor recurrence. 24

6. Conclusion

In recent years, CAR T cell immunotherapy has achieved great successes and progresses in treating leukemia [27]. But many questions, such as the toxicity, high rate of relapse and limited efficacy in

solid tumors, still remain to be solved [28]. To address all these questions, novel design of CAR T cells must be further developed and created. In this review, we summarized the recent development of CAR T cells. Of them, many novel CAR design greatly improved the activity, proliferation and persistence of CAR T cells. The reliable, safe and effective CAR T cells are the premise to clinical use, and is also the goal of tumor immunologist, we expect the application of CAR T therapy to a broad range of tumors in the near future.

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